Calcium: a fundamental regulator of intracellular membrane fusion?

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For many years, it has been known that an increase in cytosolic calcium triggers the fusion of secretory granules and synaptic vesicles with the plasma membrane. However, the role of calcium in the intracellular membrane-fusion reactions that coordinate the secretory and endocytic pathways has been less clear. Initially, there was accumulating evidence to indicate that a focally localized and transient calcium signal is required to trigger even those fusion events formerly classified as 'constitutive'—that is, those that normally occur in the absence of global cytosolic calcium increases. Therefore, calcium seemed to be a required fundamental co-factor underlying *all* biological membrane-fusion steps, perhaps with a conserved mechanism of action. However, although such unification would be gratifying, new data indicate that several intracellular fusion events do not require calcium after all. In this review, the evidence for calcium requirements and its modes of action in constitutive trafficking are discussed. As a challenging perspective, I suggest that the specific absence of calcium requirements for some transport steps in fact *expands* the function of calcium in trafficking, because divergent luminal calcium concentrations and requirements for fusion might increase the specificity with which intracellular membrane-fusion partners are determined.

Introduction

Vesicle targeting and fusion in the endomembrane system are mediated by several conserved protein families. Those that are relevant to this review are the vesicle coat–protein complexes, such as COPI and COPII, which select cargo and catalyse the budding of transport vesicles with specific compositions and destinations. Rab GTPases coordinate tethering interactions between vesicles and target membranes, and activate the fusion machinery. Soluble N-ethylmaleimide-sensitive factor attachment protein receptors

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(SNAREs) are integral membrane proteins that bridge the opposing membranes and mediate their fusion.

Calcium is required in constitutive membrane trafficking

The first indications that calcium is required for constitutive secretion came from studies of trafficking from the endoplasmic reticulum (ER) to the Golgi (Beckers & Balch, 1989). Subsequently, calcium was shown to be required for early endosome fusion (Colombo *et al*, 1997) and yeast homotypic vacuole fusion (Peters & Mayer, 1998). In these cases, fusion was inhibited by the fast calcium chelator 1,2-bis(2-aminophenoxy)ethane-N,N,N′,N′-tetraacetic acid (BAPTA), but not by the slower chelator of comparable calcium affinity, ethylene glycol-bis(2-aminoethylether)-N,N,N′,N′-tetraacetic acid (EGTA). A 10 mM solution of BAPTA would take 0.3 µs to quench a typical cellular calcium gradient, whereas EGTA at the same concentration would take 1.2 ms (Adler *et al*, 1991). These observations, together with the selectivity of inhibition by BAPTA, led to the conclusion that elevated calcium levels are required for less than 1 ms. In a recent review, Burgoyne and Clague argue that given the diffusion rate of calcium in the cytosol, a less than 1 ms transient calcium gradient would dissipate within approximately 20 nm and, therefore, that the source of the calcium gradient—the release channel—must be within approximately 20 nm of the membrane-fusion apparatus (Burgoyne & Clague, 2003). This tight temporal and spatial coupling would be possible because the source of the required calcium seems to be the lumen of the fusion partners themselves, and the depletion of luminal calcium stores mimics the effect of BAPTA (Holroyd *et al*, 1999; Peters & Mayer, 1998). A model therefore emerged in which successful docking of the vesicles through the tether–SNARE machinery activates the release of luminal calcium; this calcium 'signal' then triggers membrane fusion by activating calcium-binding proteins that regulate the fusion apparatus. Similar studies to those described above implicated transient luminal calcium release as a requirement for mammalian intra-Golgi transport (Porat & Elazar, 2000) and endosome–lysosome fusion (Pryor *et al*, 2000). Note that the requirement for a short pulse of elevated calcium does not require the membrane-fusion event to be comparably fast, as calcium effector proteins might remain active for some time after dissipation of the gradient.

The perceived universality of a role of calcium in fusion peaked around the year 2002, when an informed observer would not have been able to identify a single vesicle-transport step for which a calcium
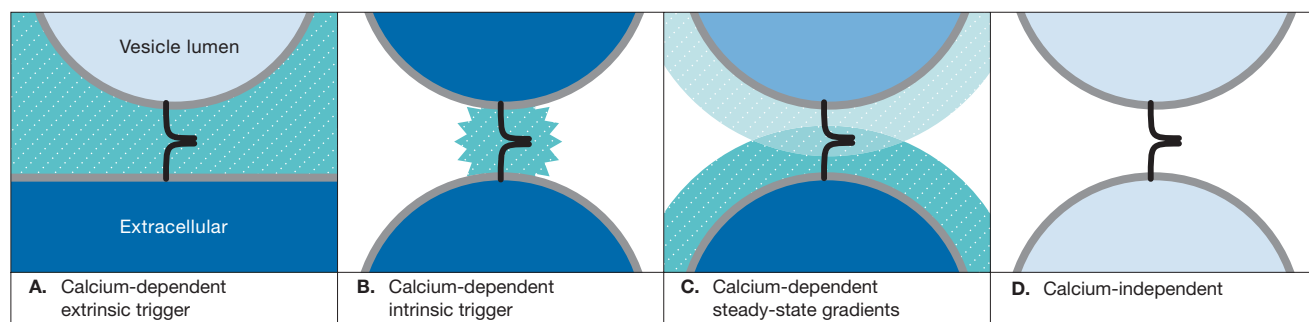
Fig 1 | Models of calcium signalling in membrane fusion. Blue represents luminal and extracelluar calcium, with lighter shades representing lower concentrations. Blue-green represents cytosolic calcium. **(A)** Calcium-dependent with extrinsic trigger. Global or focally restricted cytosolic calcium elevation owing to cell signalling events triggers membrane fusion, for example in exocytosis. **(B)** Calcium-dependent with intrinsic trigger. Completion of docking and/or soluble N-ethylmaleimide-sensitive factor attachment protein receptor (SNARE) pairing triggers a transient, localized release of calcium from the lumens of the paired membranes, activating the fusion machinery. This model has been proposed for numerous 'constitutive' fusion events. **(C)** Calcium-dependent regulation by steady-state calcium gradients. Constantly leaking luminal calcium regulates transport machinery. Different luminal calcium concentrations and/or leak rates of different organelles could differentially modulate processes, for example, coat-protein complex I (COPI)/COPII vesicle coating/uncoating. **(D)** Calcium-independent. Calcium is apparently not required at some steps, for example, yeast vacuole fusion and COPII vesicle/Golgi fusion. In some cases (not pictured), a calcium requirement could exist but be met by resting cytosolic calcium concentrations; in this scenario, basal cytosolic calcium would be a permissive co-factor but not a trigger or regulator.

requirement had not been established. It therefore seemed that there were just two kinds of biological membrane fusions (Fig 1): those that required a calcium trigger initiated by *extrinsic* signalling events, for example, calcium-regulated exocytosis, and those that required a calcium trigger initiated by *intrinsic* signalling events, for example, ER-to-Golgi transport and endosome fusion.

Several trafficking steps do not require calcium

The tides began to turn against the concept of calcium as a fundamental trigger for fusion when Stamnes and colleagues added membrane-permeant chelators to intact, living cells (Chen *et al*, 2002). Their study used mostly morphological assessment of cargo transport through the endomembrane system to test whether BAPTA-AM, which is the membrane-permeant acetoxymethyl ester of BAPTA, blocked constitutive transport at various steps. Interestingly, although calcium chelation inhibited both anterograde and retrograde transport through the secretory pathway, certain steps seemed conspicuously unaffected. For example, cargo transport from the ER to the ER-Golgi intermediate compartment (ERGIC)—also known as the vesicular tubular clusters (VTCs)—was not inhibited, whereas traffic from the VTCs to the Golgi was blocked. Similarly, transport through the Golgi was blocked, whereas no accumulation point between the Golgi and the plasma membrane could be observed. In the retrograde pathway, endocytosis itself was not affected by calcium chelation, whereas endosome-to-Golgi and Golgi-to-ER trafficking were blocked. These data indicate that the constitutive secretory pathway might be a mosaic of calcium-dependent and calcium-independent transport steps. The lack of effect on exocytosis might be explained by the technical difficulty of getting the chelator into cells at just the right time to arrest carriers between the Golgi and plasma membrane. However, the lack of effect on ER-to-VTC transport cannot be similarly explained, as the chelator was added before cargo release from the ER. An alternative explanation is that ER-to-VTC transport might not involve vesicles. Indeed, VTCs have been suggested to be directly extruded *en bloc* from the ER. However,

the existence of approximately 50 nm cargo-containing COPII vesicles emanating from ER-exit sites has now been established using immuno-electron tomography (Zeuschner *et al*, 2006). Furthermore, COPII vesicles have been shown to undergo homotypic fusion, providing a pathway for *de novo* VTC generation and ER-to-VTC cargo transport by a traditional vesicle-transport mechanism (Xu & Hay, 2004). Importantly, unlike the fusion of pre-Golgi intermediates with the Golgi, which is inhibited by chelators, the homotypic fusion of COPII vesicles *in vitro* to form pre-Golgi intermediates is not inhibited by either BAPTA or EGTA, exactly as would be predicted by the 'mosaic' interpretation of the Stamnes study (M. Bentley & J.C.H., unpublished data). Therefore, there is compelling evidence from both *in vitro* biochemical and intact-cell studies that the first membrane-fusion step in the secretory pathway—COPII vesicle fusion—does not require calcium, whereas the second fusion step—between the VTCs and the Golgi—does.

Recently, two other studies using biochemical reconstitutions of transport processes have challenged previously established requirements for calcium in constitutive membrane-fusion steps. A study from the Wickner group (Starai *et al*, 2005) on yeast vacuole fusion challenges much of the earlier work, implying that transiently released luminal calcium is required to trigger the last stages of membrane fusion (Peters & Mayer, 1998). The more recent study shows that BAPTA inhibition of vacuole fusion can be accounted for, in part, by its effects on ionic strength. Vacuole fusion is sensitive to ionic strength and, under normal conditions (100 mM KCl), the tetravalent BAPTA ion inhibits fusion simply by extracting required peripheral membrane proteins. Importantly, however, even under low ionic strength conditions in which BAPTA *does* seem to have an inhibitory role primarily through chelation of free calcium, the 2005 study shows that this function is not *unique* to calcium and that it can be substituted by magnesium. This is inconsistent with a calcium-specific effector mechanism involving calcium-binding proteins and instead implies that divalent cations have a more direct, 'biophysical' role in favouring

membrane transformations, such as lipid-stalk or fusion-pore formation. This type of role has been characterized in pure lipid-fusion studies, but had not been implicated in Rab-dependent and SNARE-dependent biological fusion reactions. This work implies that, although divalent cations might have an important general role, there is no specific and required signalling role for calcium in vacuole fusion.

A second paper has argued that the calcium requirement for fusion of yeast COPII vesicles with the Golgi is actually a requirement of the content-mixing assay, rather than the membrane-fusion event itself (Flanagan & Barlowe, 2006). The Barlowe group used *cis*-SNARE-complex formation by two SNAREs originally present on the COPII vesicle and the Golgi, respectively, to measure the extent of content mixing between the two compartments that coincided with fusion. The results of this new fusion assay were compared with those obtained using the 'traditional' content-mixing assay; the latter relies on Golgi-specific carbohydrate modification of the cargo glycoprotein α-factor as a readout of fusion between COPII vesicles containing the unmodified α-factor and Golgi membranes containing the mannosyltransferase. Surprisingly, the calcium chelators EGTA and BAPTA, which completely block the glycosylation-based assay, had no impact on fusion as measured with *cis*-SNARE complexes. Furthermore, the block caused by chelators could be completely rescued by manganese, but not calcium, re-addition. Together with the known requirement of Golgi mannosyltransferases for manganese, this probably means that the observed inhibition of 'fusion' was, in fact, an artefact of manganese chelation by EGTA and BAPTA. This study therefore shows, as do the former examples, that a SNARE-dependent and Rab-dependent membrane-fusion process can proceed normally without a detectable requirement for even basal levels of calcium. This study does not necessarily argue against the calcium requirement shown for mammalian ER-to-Golgi transport, in which chelator-induced transport blocks have been observed morphologically (for example, in the Stamnes study described above) as well as biochemically, numerous times. Rather than dismiss all chelator studies of constitutive transport, it will be important to continue testing the involvement of particular calcium pumps, release channels, calcium sensors and effectors, by using both genetic and biochemical approaches.

Calcium mechanisms of action

Synaptotagmins are calcium sensors that are believed to mediate the calcium dependency of regulated exocytosis (Tang *et al*, 2006). These molecules, however, are mostly absent from the early secretory pathway and are unlikely to be involved in calcium regulation of constitutive transport steps. Most attention concerned with how calcium might affect membrane fusion in constitutive transport steps has been focused on the calcium-binding protein calmodulin. Calmodulin antagonists have implicated this effector in endosome fusion and intra-Golgi vesicle transport, yeast vacuole fusion (but, see above) and exocytosis, in which calmodulin seems to have a modulatory, but not an essential, role. Several interesting possible mechanisms of action have emerged, which are only briefly summarized here (see the review by Burgoyne & Clague, 2003 for more details). First, calmodulin has been shown to interact with several SNAREs and vesicle tethers in a calcium-dependent manner; these include vesicle-associated membrane protein 2 (VAMP2; De Haro *et al*, 2003), which is involved in exo/endocytosis, and syntaxin 13 and early endosome antigen 1 (EEA1; Mills *et al*, 2001), which

participate in the early endosomal tethering–fusion complex. Evidence has also been presented that calmodulin-dependent protein kinase II is an important calmodulin effector for endosome fusion (Colombo *et al*, 1997). Clearly, these are interesting observations; however, neither the function nor the mechanism of action of calmodulin has been resolved for any membrane-fusion step.

Another calcium-sensing protein, Hrs, illustrates a general mechanism by which luminal calcium can affect the activity of SNAREs in endosome fusion. Hrs is a SNARE-mimetic protein—meaning that it directly interacts with SNAREs through a 'SNARE-like' coiled-coil, but inhibits membrane fusion by occupying the position normally held by a fusogenic SNARE. Hrs is a large, multifunctional peripheral membrane protein that interacts with the Q-SNARE SNAP-25. The addition of purified Hrs to an *in vitro* early endosome-fusion assay inhibited membrane fusion, apparently by interacting with the SNAP-25–syntaxin 13 Q-SNARE complex and inhibiting VAMP2 binding (Sun *et al*, 2003). Interestingly, calcium reversed the SNAP-25–Hrs interaction and inhibition (Yan *et al*, 2004). These data suggest a model in which Hrs prevents SNARE-complex formation until the appropriate signal—calcium efflux from the endosome lumen—reverses the inhibition and allows *trans*-SNARE-complex formation. It seems likely that at least part of the requirement for calcium in endosome fusion reflects the requirement to release the SNAREs from Hrs.

Other calcium effectors in the constitutive secretory pathway include the coat proteins responsible for transport-vesicle formation. Stamnes and colleagues observed that membrane-permeant BAPTA-AM caused an apparent redistribution of COPI from Golgi membranes to the cytosol (Ahluwalia *et al*, 2001). Inspection of *in vitro* COPI-vesicle budding and binding assays revealed that BAPTA destabilized COPI binding to membranes, and could block vesicle budding or elicit the uncoating of already-budded COPI vesicles. Interestingly, BAPTA had a much greater effect than EGTA, mirroring the inhibitory effects on transport assays and implying that the requirement for luminal calcium in transport might relate to coat dynamics. An exciting new development is the discovery that the COPII coat is also stabilized on the membrane by calcium. Komada and colleagues have discovered that the penta-EF-hand-containing protein apoptosis-linked gene 2 (ALG-2) acts as a calcium sensor on the membrane of ER-exit sites and, when calcium is present, stabilizes the association of the sec31 COPII subunit with the membrane (Yamasaki & Komada, 2006). Although the functional significance of the COPII calcium regulation is not yet clear, this study has identified a calcium sensor–effector mechanism by which luminal calcium might influence a constitutive transport step. The calcium sensor for COPI retention on the Golgi is not known. One potentially interesting suggestion is the discovery of an interaction between neuronal calcium sensor-1 (NCS-1), which is a peripheral membrane EF-hand-containing protein, and Arf1, which is a regulatory GTPase that controls COPI-vesicle biogenesis. Although there is strong evidence that Arf1 and NCS-1 interact at the *trans*-Golgi network (Haynes *et al*, 2005), the precise functional relevance of the interaction to vesicle biogenesis and trafficking remains to be seen.

How would calcium-dependent COPI/II coat stabilization affect transport? The calcium-dependent coat stabilization might be required to initially produce the vesicles themselves or, alternatively, to protect them from immediate back-fusion with the donor cisternae (Ahluwalia *et al*, 2001). A later role in directly regulating fusion could also be imagined, as tethered vesicles seem to retain

significant coat proteins (Cai *et al*, 2007). Note that the regulation of coat proteins by calcium does not necessarily translate into a *positive* role for calcium in fusion—the direction of the effect on trafficking is hard to predict as calcium stabilization of coats could translate into an inhibition of back-fusion with donor membranes and therefore *promote* vesicle formation, or it could *inhibit* fusion with target membranes. The ultimate effects of calcium regulation of coats could differ at different steps.

Assuming that luminal calcium release is important for intracellular membrane fusion, what would be the release/triggering mechanism? One possibility is that calcium-release channels might be functionally coupled to the vesicle-docking machinery, such that formation of a membrane-bridging docking complex could activate localized calcium release. In neurotransmission, there is growing evidence that syntaxin 1, SNAP-25 and other components of the trafficking machinery interact directly with plasma membrane voltage-gated calcium channels, and modulate their release activity and synaptic calcium dynamics (Jarvis & Zamponi, 2005; Verderio *et al*, 2004). The results are consistent with a model in which unassembled SNAREs inhibit calcium influx, and SNARE-complex assembly releases the inhibition. The interaction with the SNARE machinery is also expected to restrict the site of membrane fusion to near the origin of the calcium gradient. A similar interaction between the fusion machinery and calcium-release channels might explain how constitutive fusion steps could be triggered by docking-dependent luminal calcium release (Merz & Wickner, 2004). An interesting, but so far functionally uncharacterized, interaction between snapin—a SNARE regulatory protein—and the ryanodine-sensitive calcium channel has been recently documented (Zissimopoulos *et al*, 2006).

A different view of the calcium-release mechanism for constitutive fusion events is implied by the accumulating evidence that calcium regulates COPI/COPII coat dynamics (see above). It is harder to imagine a transient or spatially restricted calcium signal involved in coat assembly/disassembly. However, a constant, slow leakage of calcium from the ER and/or Golgi cisternae could create a perpetual calcium gradient surrounding these organelles, which could regulate coat dynamics on the membrane. Standing calcium gradients surrounding secretory organelles at steady-state have, indeed, been indicated by using calcium-imaging techniques (Wahl *et al*, 1992). Assuming that the requirement for calcium in constitutive membrane traffic is due to events close to the calcium-release channel, it is possible to justify the preferential effects of BAPTA over EGTA in the context of a steady-state calcium 'leak' gradient. Within a short distance of the release channel (approximtely 20 nm according to the Burgoyne estimate, see above), EGTA buffering of a cellular calcium gradient arising from an open channel would never reach equilibrium while the channel is open. This means that calcium-dependent processes localized nearby could experience constantly elevated calcium, even in the presence of EGTA. As BAPTA buffering is so much faster, the 'halo' of elevated calcium levels surrounding a leaking channel in the presence of BAPTA would simply be too small to include the relevant protein machinery. Close to calcium-leak channels, constitutively released luminal calcium might regulate 'slow' calcium-dependent processes, such as vesicle coating/uncoating or constitutive membrane fusion. This scheme is different from the original concept of a fast pulse of calcium triggered by events intrinsic to the transport machinery itself. Would it constitute a 'signalling' as opposed to a 'basal' role for calcium?

Alterations of the calcium halo as vesicles matured and approached other organelles would potentially define coating and uncoating zones, and, on tethering, the fusion machinery on each membrane could sense calcium released by the other membrane. Leaking calcium could therefore provide spatial cues, as well as potentially encoding biochemical compatibility for the fusion of membrane partners (see below); this would qualify as a signalling role for calcium. The regulation of transport machinery by steady-state calcium gradients is depicted in Fig 1.

Unfortunately, little or no progress has been made in identifying the release mechanism for luminal calcium. Basal calcium leak from secretory organelles displays properties indicative of an ion-conducting channel; however, it remains debatable as to which are the most significant proteins responsible. Basal calcium leak is mediated, at least in part, in the ER and Golgi by inositol trisphosphate receptor channels, and in the ER by ribosome-bound translocons after their release of nascent polypeptides. Another possible leak mechanism is back-flow through the sarco-endoplasmic reticulum calcium ATPase (SERCA) calcium pump itself (for a detailed discussion, see Camello *et al*, 2002).

Summary and perspectives

After an uncertain beginning, studies of calcium regulation in the constitutive secretory pathway have finally begun to take some incisive steps forward, as well as some unforeseen twists. Clearly, calcium does not seem to be a fundamental co-factor or trigger for biological membrane fusion. However, there do appear to be a number of constitutive transport steps—for example, endosome fusion, and VTC-to-Golgi and intra-Golgi transport—in which luminal calcium might yet have required or important regulatory roles. Rather than viewing calcium as a fundamental regulator of membrane transport, it might be more appropriate to view it as potentially regulating transport in fundamentally different ways. The regulatory effects of calcium might be superimposed in a piecemeal fashion over the Rab-dependent and SNARE-dependent constitutive fusion machinery, with the mechanism and sensitivity of the regulation 'customized' for the specific ionic luminal environments and ion permeabilities of the particular membranes involved at each transport step. Furthermore, the specific requirement for calcium at some, but not all, membrane-fusion steps could actually help encode specificity in the secretory pathway by synchronizing the membrane-fusion machinery with the luminal environment of its intended targets. This is exemplified hypothetically below in the case of ER-to-Golgi transport, which represents a complicated membrane interface involving several ordered membrane-fusion steps as well as membrane-maturation processes.

In mammalian ER-to-Golgi transport, at least two membrane-fusion steps have been defined experimentally: a pre-Golgi fusion among COPII vesicles and VTCs, which is not inhibited by chelators; and a fusion of mature VTCs with the Golgi, which is blocked by chelators (see above). Both the ER and the Golgi are major sources of releasable luminal calcium. Conversely, VTCs seem to be depleted of luminal calcium (Pezzati *et al*, 1997). If it is true that releasable calcium markedly decreases from the ER to the VTCs, and then markedly increases from the VTCs to the Golgi, it is easy to see how calcium could be used to impart directionality to this series of membrane fusions. The pre-Golgi membrane fusion should not require calcium (or could even be inhibited by it) because the intended target membranes for COPII vesicles—VTCs and other

COPII vesicles—seem to contain limited releasable calcium, whereas the off-target fusion partners—the ER and Golgi—contain abundant releasable calcium. A calcium requirement at this membrane-fusion step would have the deleterious consequence of favouring back-fusion with the ER or premature fusion with the Golgi, circumventing the sorting and quality-control steps performed by VTCs. Conversely, the later ER–Golgi membrane-fusion step—between mature VTCs and the Golgi—*should* require calcium, because the intended target membrane, the Golgi, contains abundant releasable calcium, but off-target immature VTCs do not. In this case, a calcium requirement would favour the most productive fusion partnering, whereas in the earlier fusion event it would have favoured the least productive partnering.

This additional layer of specificity might be important at transport axes, like ER-to-Golgi, or endosomes, which seem to involve gradual changes in protein composition as the transport intermediates mature, and the membranes involved contain highly overlapping sets of Rabs and SNAREs. The luminal ionic environment of the membranes involved might therefore be an important determinant in the membrane-fusion compatibility matrix. Differing calcium requirements for the fusion of membranes with similar compositions of SNAREs, coats and GTPases would require the differential recruitment of calcium sensors and effector proteins. We are only just beginning to discover the identities of these components.

REFERENCES
Adler EM, Augustine GJ, Duffy SN, Charlton MP (1991) Alien intracellular calcium chelators attenuate neurotransmitter release at the squid giant synapse. *J Neurosci* **11**: 1496–1507

Ahluwalia JP, Topp JD, Weirather K, Zimmerman M, Stamnes M (2001) A role for calcium in stabilizing transport vesicle coats. *J Biol Chem* **276**: 34148–34155

Beckers CJ, Balch WE (1989) Calcium and GTP: essential components in vesicular trafficking between the endoplasmic reticulum and Golgi apparatus. *J Cell Biol* **108**: 1245–1256

Burgoyne RD, Clague MJ (2003) Calcium and calmodulin in membrane fusion. *Biochim Biophys Acta* **1641**: 137–143

Cai H, Yu S, Menon S, Cai Y, Lazarova D, Fu C, Reinisch K, Hay J, Ferro-Novick S (2007) TRAPPI tethers COPII vesicles by binding the coat subunit Sec23. *Nature* **445**: 941–944

Camello C, Lomax R, Petersen OH, Tepikin AV (2002) Calcium leak from intracellular stores: the enigma of calcium signalling. *Cell Calcium* **32**: 355–361

Chen JL, Ahluwalia JP, Stamnes M (2002) Selective effects of calcium chelators on anterograde and retrograde protein transport in the cell. *J Biol Chem* **277**: 35682–35687

Colombo MI, Beron W, Stahl PD (1997) Calmodulin regulates endosome fusion. *J Biol Chem* **272**: 7707–7712

De Haro L, Quetglas S, Iborra C, Leveque C, Seagar M (2003) Calmodulin-dependent regulation of a lipid binding domain in the v-SNARE synaptobrevin and its role in vesicular fusion. *Biol Cell* **95**: 459–464

Flanagan JJ, Barlowe C (2006) Cysteine-disulfide cross-linking to monitor SNARE complex assembly during endoplasmic reticulum–Golgi transport. *J Biol Chem* **281**: 2281–2288

Haynes LP, Thomas GM, Burgoyne RD (2005) Interaction of neuronal calcium sensor-1 and ADP-ribosylation factor 1 allows bidirectional control of phosphatidylinositol 4-kinase β and *trans*-Golgi network-plasma membrane traffic. *J Biol Chem* **280**: 6047–6054

Holroyd C, Kistner U, Annaert W, Jahn R (1999) Fusion of endosomes involved in synaptic vesicle recycling. *Mol Biol Cell* **10**: 3035–3044

Jarvis SE, Zamponi GW (2005) Masters or slaves? Vesicle release machinery and the regulation of presynaptic calcium channels. *Cell Calcium* **37**: 483–488

Merz AJ, Wickner WT (2004) *Trans*-SNARE interactions elicit Ca^{2+} efflux from the yeast vacuole lumen. *J Cell Biol* **164**: 195–206

Mills IG, Urbe S, Clague MJ (2001) Relationships between EEA1 binding partners and their role in endosome fusion. *J Cell Sci* **114**: 1959–1965

Peters C, Mayer A (1998) Ca^{2+}/calmodulin signals the completion of docking and triggers a late step of vacuole fusion. *Nature* **396**: 575–580

Pezzati R, Bossi M, Podini P, Meldolesi J, Grohovaz F (1997) High-resolution calcium mapping of the endoplasmic reticulum-Golgi-exocytic membrane system. Electron energy loss imaging analysis of quick frozen-freeze dried PC12 cells. *Mol Biol Cell* **8**: 1501–1512

Porat A, Elazar Z (2000) Regulation of intra-Golgi membrane transport by calcium. *J Biol Chem* **275**: 29233–29237

Pryor PR, Mullock BM, Bright NA, Gray SR, Luzio JP (2000) The role of intraorganellar Ca^{2+} in late endosome-lysosome heterotypic fusion and in the reformation of lysosomes from hybrid organelles. *J Cell Biol* **149**: 1053–1062

Starai VJ, Thorngren N, Fratti RA, Wickner W (2005) Ion regulation of homotypic vacuole fusion in *Saccharomyces cerevisiae*. *J Biol Chem* **280**: 16754–16762

Sun W, Yan Q, Vida TA, Bean AJ (2003) Hrs regulates early endosome fusion by inhibiting formation of an endosomal SNARE complex. *J Cell Biol* **162**: 125–137

Tang J, Maximov A, Shin OH, Dai H, Rizo J, Sudhof TC (2006) A complexin/synaptotagmin 1 switch controls fast synaptic vesicle exocytosis. *Cell* **126**: 1175–1187

Verderio C, Pozzi D, Pravettoni E, Inverardi F, Schenk U, Coco S, Proux-Gillardeaux V, Galli T, Rossetto O, Frassoni C, Matteoli M (2004) SNAP-25 modulation of calcium dynamics underlies differences in GABAergic and glutamatergic responsiveness to depolarization. *Neuron* **41**: 599–610

Wahl M, Sleight RG, Gruenstein E (1992) Association of cytoplasmic free Ca^{2+} gradients with subcellular organelles. *J Cell Physiol* **150**: 593–609

Xu D, Hay JC (2004) Reconstitution of COPII vesicle fusion to generate a pre-Golgi intermediate compartment. *J Cell Biol* **167**: 997–1003

Yamasaki A, Komada M (2006) The Ca-binding protein ALG-2 is recruited to ER exit sites by sec31A and stabilizes the localization of sec31A. *Mol Biol Cell* **17**: 4876–4887

Yan Q, Sun W, McNew JA, Vida TA, Bean AJ (2004) Ca^{2+} and N-ethylmaleimide-sensitive factor differentially regulate disassembly of SNARE complexes on early endosomes. *J Biol Chem* **279**: 18270–18276

Zeuschner D, Geerts WJ, van Donselaar E, Humbel BM, Slot JW, Koster AJ, Klumperman J (2006) Immuno-electron tomography of ER exit sites reveals the existence of free COPII-coated transport carriers. *Nat Cell Biol* **8**: 377–383

Zissimopoulos S, West DJ, Williams AJ, Lai FA (2006) Ryanodine receptor interaction with the SNARE-associated protein snapin. *J Cell Sci* **119**: 2386–2397

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